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REVIEW ARTICLE

THE TRANSITION FROM HYPERPLASIA TO INVASIVE CARCINOMA OF THE BREAST

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SUMMARY

The multistep model of carcinogenesis in the breast suggests a transition from normal epithelium to invasive carcinoma via non-atypical and atypical hyperplasia and *in situ* carcinoma. Within the breast, these proliferations are heterogeneous in their cytological and architectural characteristics. This review considers the evidence supporting a precursor role for these preinvasive lesions. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS—**breast; preinvasive lesions; molecular genetics; multistep model; loss of heterozygosity; comparative genomic hybridization**

INTRODUCTION

Approximately one in twelve women will develop breast cancer in their lifetime. Despite major diagnostic and therapeutic innovations, the effect on mortality has been modest. One of the factors contributing to this limited success is our relative lack of understanding about the natural history of the disease. The introduction of mammographic screening has led to the increased detection of preinvasive disease, particularly ductal carcinoma *in situ* (DCIS).[1,2] The identification of preinvasive disease and, in particular, 'borderline lesions' has highlighted deficiencies in our understanding and classification of such lesions within the breast. The morphological classification of breast disease remains controversial and difficulties are encountered in the subclassification of DCIS, differentiating DCIS from atypical ductal hyperplasia (ADH), and differentiating low nuclear grade (LNG) DCIS of solid type from lobular carcinoma *in situ* (LCIS).

THE MULTISTEP MODEL FOR BREAST CARCINOGENESIS

The hypothetical multistep model for carcinogenesis within the breast (Fig. 1) indicates that invasive carcinoma arises via a series of intermediate hyperplastic (with and without atypia) and neoplastic (*in situ* carcinoma) stages. Within the colon, there is a well-defined preinvasive lesion in the form of an adenoma and this has facilitated the delineation of genetic alterations in this putative precursor lesion.[3] Studies in the breast have been complicated by the morphological heterogeneity of the preinvasive lesions. Furthermore, tissue heterogeneity, with fat, blood, lymphatic vessels, and inflammatory cells in close proximity to the duct-lobular units, means that there is a strong propensity to contamination, which affects the genetic analysis of these microscopic lesions. So, what is the evidence that any of these lesions are indeed precursors of invasive carcinoma?

EVIDENCE FOR PRECURSOR LESIONS

The evidence for the presence of putative precancerous lesions in the breast comes from three main sources: animal experiments, review of human histopathological material, and genetic analysis of putative precursor lesions in the human breast.

Animal experiments

The classical experimental system for the study of multistep neoplasia has been the mouse mammary tumour model.[4,5] Infection with the murine mammary tumour virus (MuMTV) leads to the transformation of normal epithelium to a proliferation known as hyperplastic alveolar nodules (HAN). They have limited growth potential and are not obliged to transform into malignant tumours, but when transplanted into cleared mammary fat pads, they develop into tumours more frequently than normal breast tissue. In experiments carried out by DeOme *et al.*,[4] 9/19 HAN transformed into carcinoma by 13–21 weeks, compared with 2/19 normal tissues after 24 weeks' follow-up. These animal experiments provided the first direct evidence for the evolution of breast cancer through intermediate stages at which progression is not inevitable, but is more likely than for normal tissue. Interestingly, recent studies suggest that HAN is clonal, indicating that it represents a neoplastic rather than a hyperplastic proliferation.[6]

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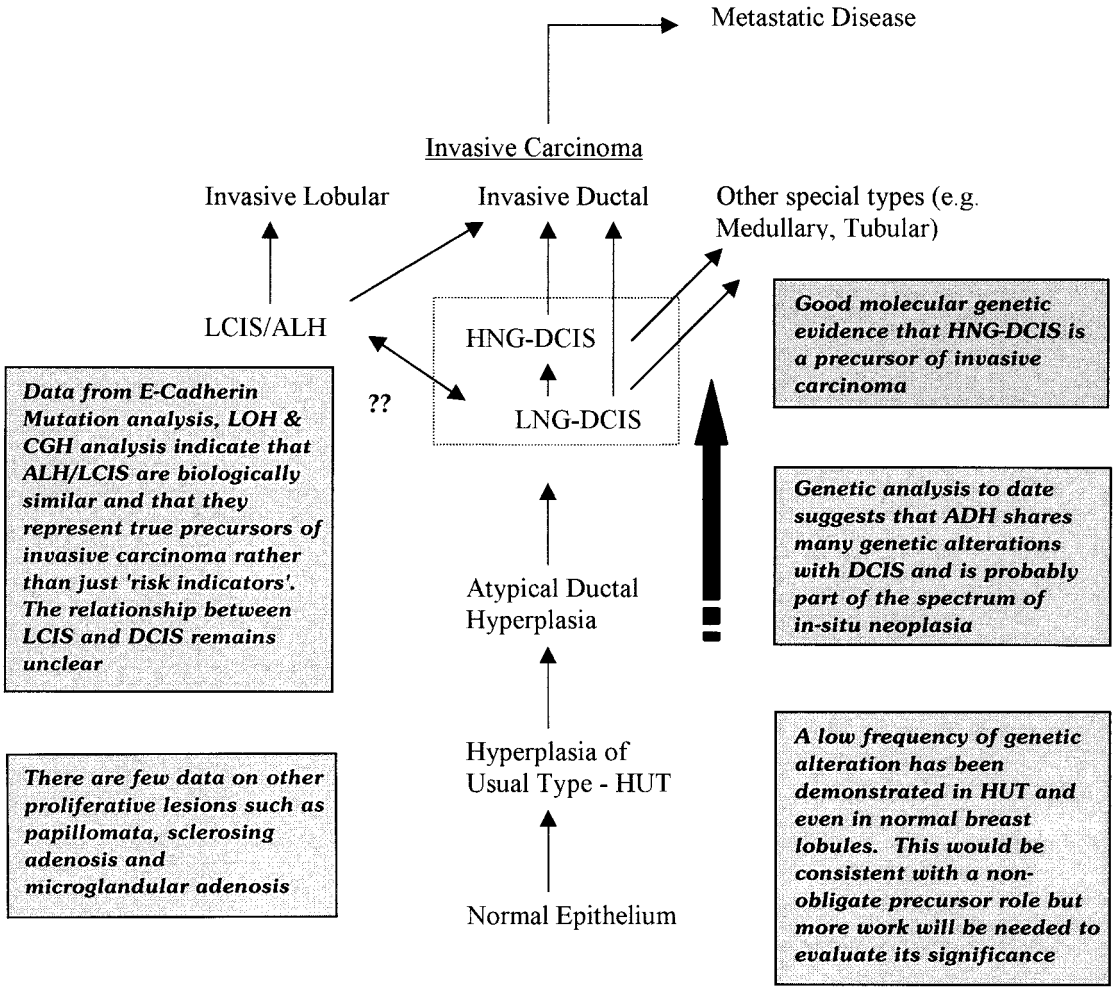

Fig. 1—Multistep model of carcinogenesis in the breast

Review of human histopathological material

These studies have provided indirect evidence for a precursor role. Four main approaches have been adopted:

(i) Identification of morphological transitions—In breast biopsies harbouring malignancy, infiltrating carcinoma is often found side-by-side with *in situ* carcinoma and/or benign proliferations. These lesions occasionally show morphological transition and continuity with the invasive carcinoma. Transitional forms between LCIS and DCIS may also be encountered in the same breast. These observations have been interpreted by histopathologists as supportive of a possible precursor role for these proliferations.

(ii) Cancerous versus non-cancerous breasts—In a comparison of 300 radical mastectomies containing malignancy with 200 partial or simple mastectomies without malignancy, Foote and Stewart[7] found that papillary hyperplasia with atypia occurred five times more frequently in the cancerous breasts. In a similar study, Ryan and Coady[8] found that hyperplasia was four times more common in the cancerous breasts. Karpas *et al.*[9] evaluated 645 breast biopsies (226 malignant and 419 benign) and found atypical hyperplasia in 62 per cent of malignant biopsies, compared with 4 per cent of benign biopsies. Similarly, Kern and Brooks[10] found a greater incidence of atypical ductal hyperplasia (ADH) in cancer-bearing breasts. Welling *et al.*[11] studied 196 whole breasts and 16 breast biopsies. In 119 specimens, quantitative morphology using 2 mm thick whole mounts was used. They found an increased incidence of atypical hyperplasia in cancerous breasts. Together, these studies provided good evidence that atypical hyperplasia is more likely to occur in breasts harbouring malignancy.

(iii) Prospective follow-up studies—Davies *et al.*[12] reviewed 20 prospective studies carried out between 1892 and 1960 in which patients with benign changes were found to develop cancer. Overall, in 284 patients described as having 'cystic breast disease' and followed for 13 years, carcinoma developed 1·7 times more frequently than expected and those in whom ductal hyperplasia was documented developed cancer 2·5 times more frequently than expected. Black *et al.*[13] found that the presence of severe atypia predicted a 5 times greater risk of developing carcinoma, compared with women with no evidence of atypia. During the 1980s, Page and

his co-workers conducted a series of important prospective studies, one of which[14] indicated that in a woman with proliferative disease, the relative risk of developing carcinoma was 1·9, rising to 5·3 if the proliferation showed evidence of atypia. Those with a positive family history of breast cancer had a relative risk of 2·7 and 11·0, respectively. Tavassoli and Norris,[15] McDivitt *et al.*,[16] and London *et al.*[17] have subsequently confirmed the increased risk associated with atypical hyperplasia.

(iv) Retrospective studies of 'benign' breast biopsies—Betsill *et al.*[18] reviewed all breast biopsies performed at Memorial Sloan Kettering Cancer Centre between 1940 and 1950. A total of 8609 biopsies were studied. Twenty-five of these were found to have untreated DCIS, of which six (24 per cent) went on to develop invasive carcinoma. The average interval for follow-up was 9·7 years. Page *et al.*[19] carried out a similar study of 11 760 breast biopsies performed between 1950 and 1968. Twenty-eight cases of DCIS were identified in this series. Of the patients followed for more than 3 years, 28 per cent (7/25) developed invasive carcinoma in the same breast as the biopsy showing DCIS. These tumours arose after an average follow-up of 6·1 years. It has to be borne in mind that the rate of subsequent carcinoma in this study may be an underestimate, as the study was heavily biased in favour of low nuclear grade DCIS. These are the lesions most likely to be mistaken for ADH or florid hyperplasia of usual type (HUT) in breast biopsies.

The histopathological studies have provided strong evidence that certain proliferative lesions within the breast are associated with an increased risk of subsequent carcinoma, supporting the hypothesis that they represent precursor lesions.

Molecular genetic analysis

(i) Invasive carcinoma—Some of the earliest indications of genetic abnormalities in breast cancer came from karyotypic studies. The literature, however, is limited due to the difficulty in culturing primary breast cancers. Although approximately 300 breast cancers have been fully karyotyped, no specific characteristic cytogenetic abnormality has been observed. The most common observation is of numerical changes to whole chromosomes. Trisomies of chromosomes 7 and 18 and complete or partial monosomies of chromosomes 6, 8, 11, 13, 16, 17, 22, and X have been recorded.[20–24] More recently, the technique of fluorescence *in situ* hybridization (FISH) has been used to catalogue chromosomal aberrations in breast cancer. Abnormalities of chromosome 1 including i(1q) and der(1;10)[25] have been reported. Comparative genomic hybridization (CGH) is a relatively new molecular-cytogenetic assay that is a modification of the FISH technique. It allows, in a single hybridization, an overview of DNA sequence copy number changes. Increased DNA sequence copy numbers have been demonstrated in breast cancer cell lines using this method.[26] Since the introduction of loss of heterozygosity (LOH) methodology by Cavenee *et al.*,[27] there have been a large number of studies investigating allelic imbalance in invasive breast carcinomas at a large number of chromosomal loci.[28] It is clear from the available data that the pattern of LOH is complex, involving numerous chromosomal locations. The chromosomal arms exhibiting LOH in excess of 25 per cent include 1p, 1q, 3p, 6q, 8p, 11q, 13q, 16q, 17p, 17q, and 22q. At the present time, only a few of the genes (e.g. *TP53*, *RB1*, *BRCA1*, *BRCA2*) have been identified and cloned and the vast majority of the putative tumour suppressor genes at these sites of LOH have not been traced. Hence we have no idea about their protein products and their functions.

***(ii) Ductal carcinoma* in situ**—DCIS forms a heterogeneous group of proliferations ranging from the low-grade cribriform type, which is difficult to differentiate from ADH, to the high-grade comedo type. The use of mammographic screening has led to an increase in the detection of DCIS and has highlighted our lack of understanding of the lesion. The classification of DCIS remains controversial and difficulties persist in distinguishing between DCIS and ADH. Cytogenetic analysis of *in situ* carcinoma has been carried out only in a small number of cases and although none has been normal, they are mainly in the diploid range. As with invasive carcinoma, abnormalities of chromosomes 1 and 16 have been identified in some of these cases.[29–33]

FISH has also been used to study chromosomal changes in DCIS. Using DNA probes to centromeric sequences on chromosomes 1, 3, 4, 6, 7, 8, 9, 10, 11, 16, 17, and 18, polysomies of chromosomes 3, 10, and 17 were identified and losses of chromosomes 1, 16, and 18 were also seen.[34] The CGH method has been modified for paraffin-embedded material, allowing studies on archival material and, in particular, the study of pre-invasive disease.[35,36] CGH analysis of DCIS has demonstrated a large number of alterations including gains of 1q, 6q, 8q, 17q, 19q, 20p, 20q, and Xq, and losses of 13q, 16q, 17p, and 22q. These alterations are similar to those identified in invasive carcinoma, adding weight to the idea that DCIS is a precursor lesion.

With the use of microdissection techniques to isolate small microscopic lesions, LOH has also been investigated in preinvasive disease. O'Connell *et al.*[37] have carried out studies on preinvasive lesions using a variety of chromosomal markers and have shown that 50 per cent of the proliferative lesions and 80 per cent of the DCIS shared their LOH patterns with invasive carcinoma. This provided the first preliminary molecular genetic evidence that these lesions are likely to be precursors of invasive carcinoma. Radford *et al.*,[38] using similar methodology, demonstrated LOH on chromosome 17p in DCIS. Recently they have produced an allelotype for DCIS,[39] but their results at certain loci are at variance with data in the literature. For instance, they found a very low incidence of LOH at chromosome 1, which is in contrast to data from Munn *et al.*[40] Stratton *et al.*[41] studied cases of DCIS associated with invasive carcinoma and cases of 'pure' DCIS without an invasive component, using a limited set of microsatellite markers on chromosomes 7q, 16q, 17p, and 17q. The sub-group

of 'pure' DCIS was important, as one of the concerns of studying DCIS in malignant breasts is that these foci represent intraductal spread of clones that are already invading stroma and hence do not represent true precursor lesions. The study demonstrated a frequency of LOH in both subsets of DCIS similar to that found in invasive carcinoma, providing further strong evidence that DCIS is likely to be a precursor lesion. Fujii *et al.*[42] have also shown LOH at 16q and 17p in 'pure' low-grade DCIS, with additional abnormalities in higher-grade DCIS lesions. Similarly, LOH in DCIS at loci on chromosome 11 has been provided by Koreth *et al.*[43] Marcello Aldaz *et al.*[44] provided a comparative allelotype of *in situ* and invasive malignancy and concluded that LOH on 1p, 3p, 3q, 6p, 16p, 18p, 18q, and 22q was not a common event in DCIS. In contrast, LOH on 7p, 16q, 17p, and 17q was observed in 25–30 per cent of DCIS.

There is a considerable body of literature on the expression of various gene products in DCIS of the breast. It is not possible to cover all the data here, but the most studied products are those of the c*erb*B2 and *TP53* genes. The proto-oncogene c*erb*B2 encodes for a tramsmembrane protein, which has homology with epidermal growth factor receptor (EGFR). Its ligand is unknown. c*erb*B2 is amplified in 20 per cent of invasive cancers and has received attention because of its association with lymph node metastases, short relapse time, poor survival, and decreased response to endocrine and chemotherapy.[45–47] c*erb*B2 amplification is almost always associated with an increase in mRNA as well as in protein expression. In contrast to invasive cancer, c*erb*B2 protein has been identified in a high proportion (60–80 per cent) of DCIS of high nuclear grade (HNG)-comedo type but is not common in the low nuclear grade (LNG) forms. Allred *et al.*[48] have shown that the expression is higher in invasive carcinomas associated with DCIS than in those without DCIS. It is very rarely expressed in LCIS.[49–51] This gene product has not been identified in benign proliferative disease or ADH.[52] The data suggest that c*erb*B2 is important in the transition from a 'benign' to a 'malignant' phenotype. The different frequency of expression in *in situ* and invasive carcinoma is a mystery. Either expression is switched off during invasion, or many c*erb*B2-positive DCIS do not transform to invasive malignancy.

p53 is a 53 kD protein that was first identified through its ability to bind and form a complex with simian virus (SV) 40 large T antigen.[53] The protein functions as a transcription factor and is involved in the control of cell proliferation. It also has a role in apoptosis. It has been demonstrated that *TP53* is the commonest molecular abnormality occurring in human cancers.[54,55] p53 protein expression has been demonstrated using immunohistochemistry in HNG-DCIS (comedo type).[56] The mechanism may be gene mutation, but this has been confirmed only in some cases.[57] Like c*erb*B2, p53 protein expression is rare in LCIS and has not been demonstrated in ADH or other benign proliferative disease.[58] Recently, Done *et al.*[59] demonstrated that p53 mutations found in DCIS and associated invasive cancer were absent from benign proliferative lesions from the same breast. Overall, there is a considerable body of evidence indicating that DCIS, particularly of high grade, shares many molecular genetic alterations with invasive carcinoma and hence is a direct precursor.

***(iii) Lobular carcinoma* in situ**—Lobular carcinoma *in situ* of the breast is an uncommon lesion with a distinctive appearance. It is composed of discohesive cells with small monomorphic hyperchromatic nuclei. It is occasionally confused with DCIS of low-grade solid type, but epidemiological studies show that it behaves quite differently from DCIS. It is usually an incidental finding and is not visible on mammography. The majority of cases are diagnosed between 40 and 50 years of age, a decade earlier than DCIS. It is also multifocal and bilateral in a high proportion of cases. Approximately one-fifth of cases progress to invasive cancer over a 25-year follow-up period and interestingly, half of these invasive cancers have a ductal phenotype. The risk is equal in both breasts. These features of LCIS have raised questions about the biological nature of LCIS, which is still generally considered to be a 'marker of increased risk' rather than a true precursor of invasive carcinoma.

Cytogenetic analysis of lobular lesions using FISH or CGH analysis has been limited. In one study using FISH,[60] 67 per cent of the cases displayed evidence of monosomy, with involvement of chromosome 17 in all six patients and chromosomes 7 and 8 in two out of six patients. Two patients with an associated invasive cancer showed trisomy for chromosomes 1 and 8 (one patient each). In our laboratories, we have recently carried out CGH analysis on LCIS and atypical lobular hyperplasia (ALH).[61] Loss of material from 16p, 16q, 17p and 22q and gain of material from 6q were found at a similar high frequency in both LCIS and ALH. Losses at 16q and 17p are also seen in invasive lobular carcinomas.[62] LOH data in LCIS are also limited. In one study,[63] LCIS has been shown to have a similar pattern of LOH to DCIS and invasive carcinoma at a number of loci (16q, 17q); at one marker, however, D17S796 on 17p in the vicinity of the *TP53* gene, the frequency of LOH in LCIS was much lower than in DCIS (8 per cent in LCIS vs. 33 per cent in DCIS). The morphological and behavioural differences between DCIS and LCIS thus also appear to be reflected at the genetic level. The similar pattern of LOH and CGH in LCIS and invasive carcinoma at other loci suggests that LCIS is also likely to be a direct precursor of invasive carcinoma rather than simply a 'risk indicator'. Further collaborative evidence comes from a study by Nayar *et al.*,[64] who studied LOH in LCIS and invasive lobular carcinoma (ILC) at two polymorphic markers for chromosome 11q13 (INT2 and PYGM). LOH was seen in approximately one-third of informative cases in both LCIS and ILC.

E-cadherin is a candidate tumour suppressor gene on 16q22.1, involved in cell–cell adhesion. Using immunohistochemistry, 50 per cent of invasive ductal carcinoma-NST have been shown to exhibit positive staining, while most invasive lobular carcinomas are negative. Recently, Berx *et al.*[65] identified protein truncation mutations in 4/7 invasive lobular carcinomas but failed to identify any

changes in 42 invasive ductal carcinoma-NST or medullary carcinomas. The mutations in the lobular tumours were accompanied by LOH in the region of the gene and absence of staining by immunohistochemistry.

E-cadherin staining has also been identified in DCIS and the molecule is expressed in normal epithelium, but staining is rarely seen in LCIS.[66,67] Recently, Vos *et al.*[68] have demonstrated the same truncating mutation in the E-cadherin gene in LCIS and the adjacent invasive lobular carcinoma. The data provide strong evidence for the role of the E-cadherin gene in the pathogenesis of lobular lesions, as well as supporting the hypothesis for a precursor role for LCIS.

(iv) Atypical ductal hyperplasia (ADH)—ADH is a controversial lesion, which shares some but not all features of DCIS. It poses considerable difficulties in surgical histopathology. In order to address this problem, Page and Rogers[69] have laid down criteria for the diagnosis of this entity. Rosai[70] had demonstrated a high inter-observer variability in the diagnosis of ADH, but a subsequent study by Schnitt *et al.*,[71] in which the pathologist used the Page criteria, showed an improvement, with complete agreement in 58 per cent of cases. Within the U.K. National External Quality Assurance Scheme,[72] agreement even amongst experienced breast pathologists has been low.

Lakhani *et al.*[73] have demonstrated that LOH identified at loci on 16q and 17p in invasive carcinoma and DCIS is also present in ADH with a similar frequency. This indicates that ADH is a neoplastic rather than a hyperplastic proliferation and should perhaps be considered within the spectrum of *in situ* ductal neoplasia. There is support for this view from a number of other studies.[37,74–76] Chuaqui *et al.*[75] demonstrated LOH in 6 of 22 (27·3 per cent) *in situ* carcinomas and in 1/11 (9 per cent) cases of ADH at 11q13. O'Connell *et al.*[76] studied 51 cases of ADH at 15 polymorphic loci and found LOH of at least one marker in 42 per cent of the cases. These studies demonstrate that at least within the limits of current molecular investigations, there is no significant difference between ADH and DCIS. The data suggest that ADH as currently defined is simply a small focus of DCIS.

(v) Hyperplasia of usual type (HUT)—HUT has also been referred to as epitheliosis and papillomatosis in the past. It may range from mild to florid proliferations and retrospective studies[15] indicate that this lesion has a relative risk of 2 for the subsequent development of invasive carcinoma.

As with ADH, cytogenetic analysis of HUT has been limited. Some studies have reported chromosomal aberrations in a proportion of HUT.[32,77] O'Connell *et al.*[76] have demonstrated that LOH at many different loci can be identified in HUT, with frequencies ranging from 0 to 15 per cent. These figures are similar to those of Lakhani *et al.*,[78] who reported data in non-atypical hyperplasia (HUT) dissected from benign breast biopsies. LOH was identified at frequencies ranging from 0 per cent at a locus on 13q (D13S267) to 13 per cent at a locus on 17q (D17S250). These frequencies are much lower than those identified in DCIS and ADH (range 25–55 per cent). The results show that at least a proportion of non-atypical hyperplasias are also clonal, neoplastic proliferations. Thus, biologically, at least some examples of HUT appear to be benign adenomas of the breast epithelium. In this study, no specific morphological features were identified that predicted allelic imbalance.

(vi) Normal tissues—Two studies over the last 2 years have also demonstrated that LOH identified in invasive carcinoma is already present in morphologically normal lobules.[79,80] The extent and frequency of changes in 'normal' tissues remain to be evaluated, but the data support the concept of multistep evolution of breast cancer.

CONCLUSION

The animal studies using the mouse mammary tumour model provide good evidence for the development of invasive carcinoma via an intermediate proliferative state—the HAN. The data from histopathological studies also provide convincing evidence that some forms of proliferative change are often found in association with invasive cancer and that ADH and DCIS provide a significantly increased relative risk of subsequent invasive carcinoma. The genetic data for a precursor role are strongest for HNG-DCIS, which shares genetic alterations with invasive carcinoma in a high proportion of cases. There are also data to indicate that ADH shows similar patterns of genetic alterations to DCIS and hence may be better regarded as lying within the spectrum of *in situ* neoplasia. Genetic data for HUT as a non-obligate precursor are at present limited, but are accumulating rapidly. The pace at which information is gathering in support of the clonal evolution of breast cancer is impressive, but many questions remain unanswered. Are the molecular events identified to date pathogenetically significant in breast cancer development? Can the genetic alterations identified be used to differentiate between the different morphological variants of preinvasive lesions and hence be useful diagnostically? What is the relationship of LCIS and ALH to DCIS and invasive cancer?

The introduction of new technology such as laser capture microdissection[81] and microarray chip analysis[82] promises to provide us with a wealth of additional molecular data over the next 5 years. With it comes the promise of typing all the protiens, RNAs, and genetic changes in normal and abnormal epithelial proliferations. Will we be able to handle all this information and will we know what to do with it?

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